Supporting information

Polycomb proteins, Ezh1 and Ezh2, co-regulate chromatin accessibility and nephron progenitor cell lifespan

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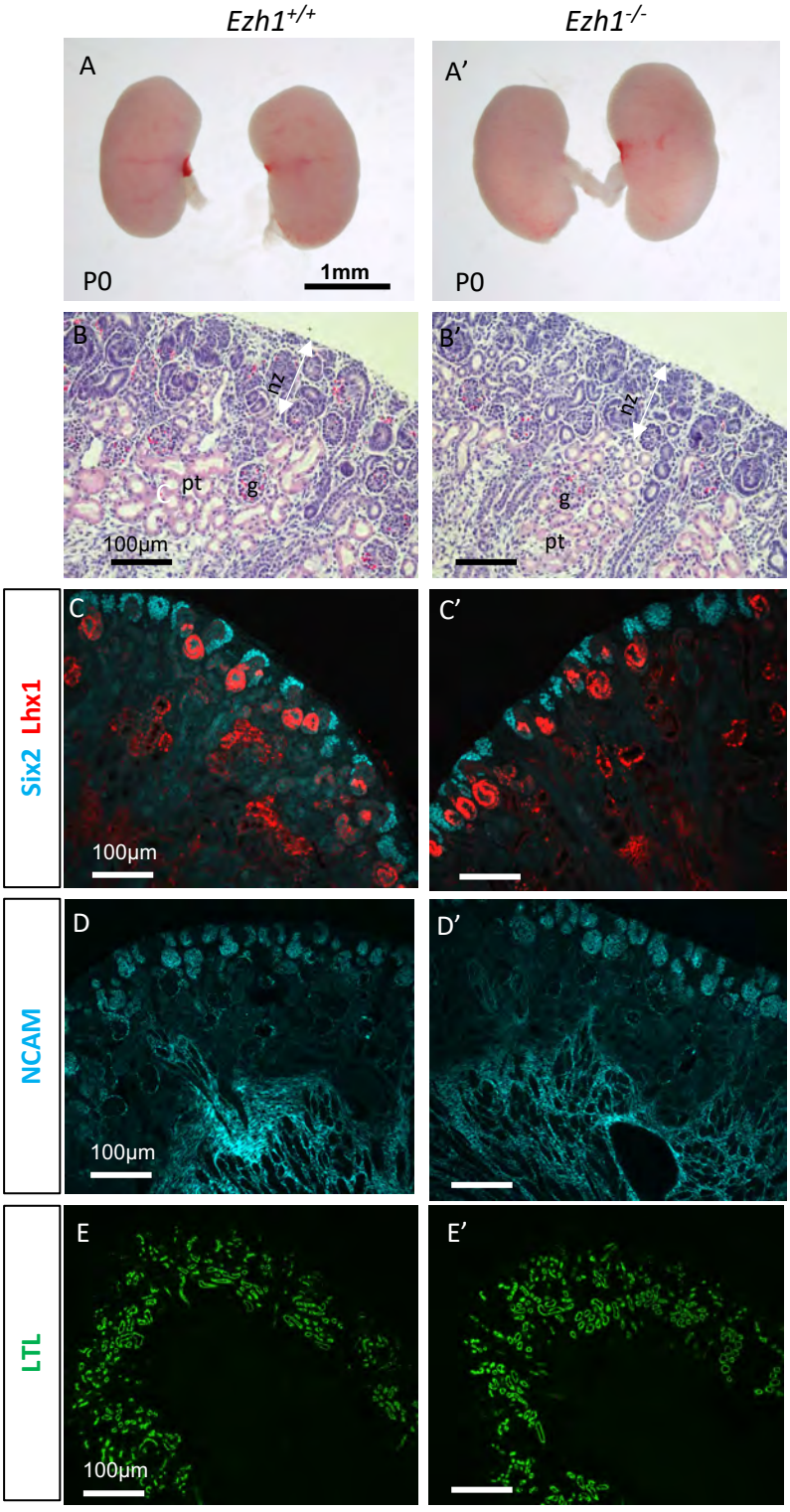
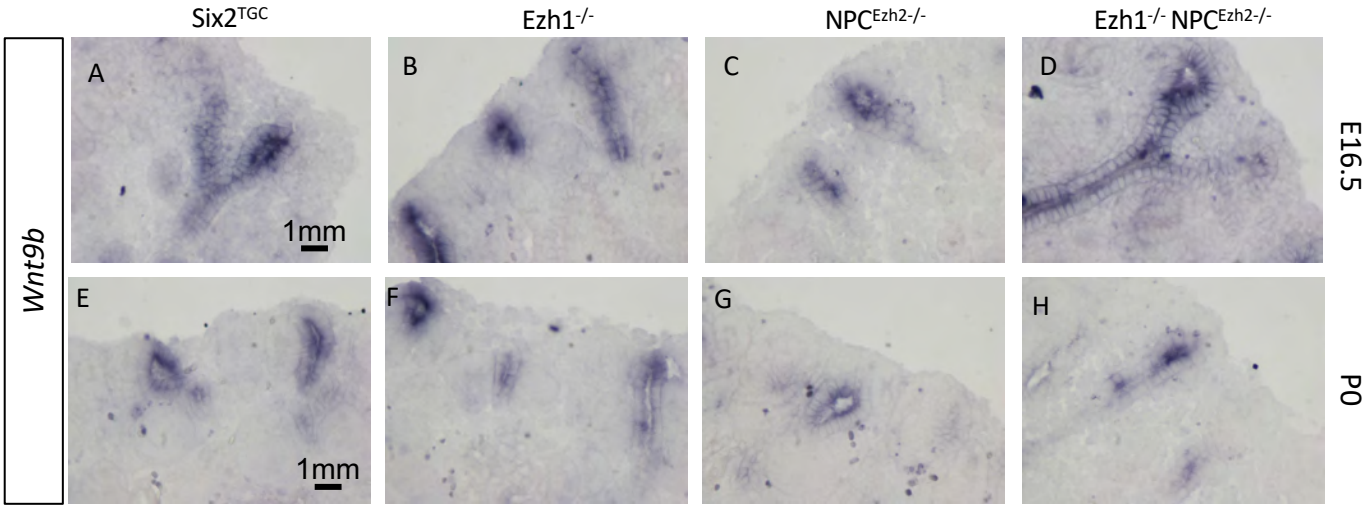
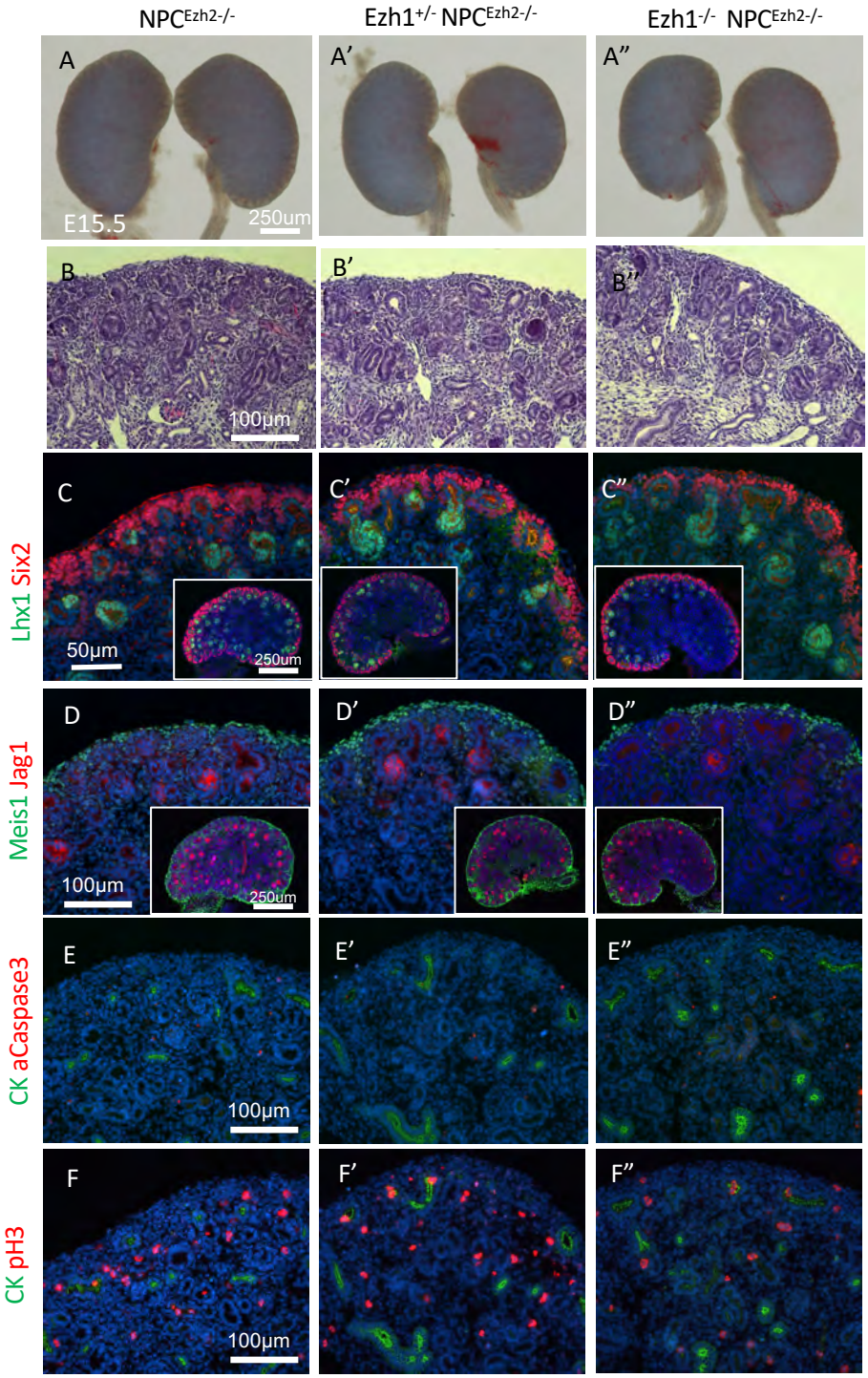
List of Material included:
Supplemental figures S1-S7



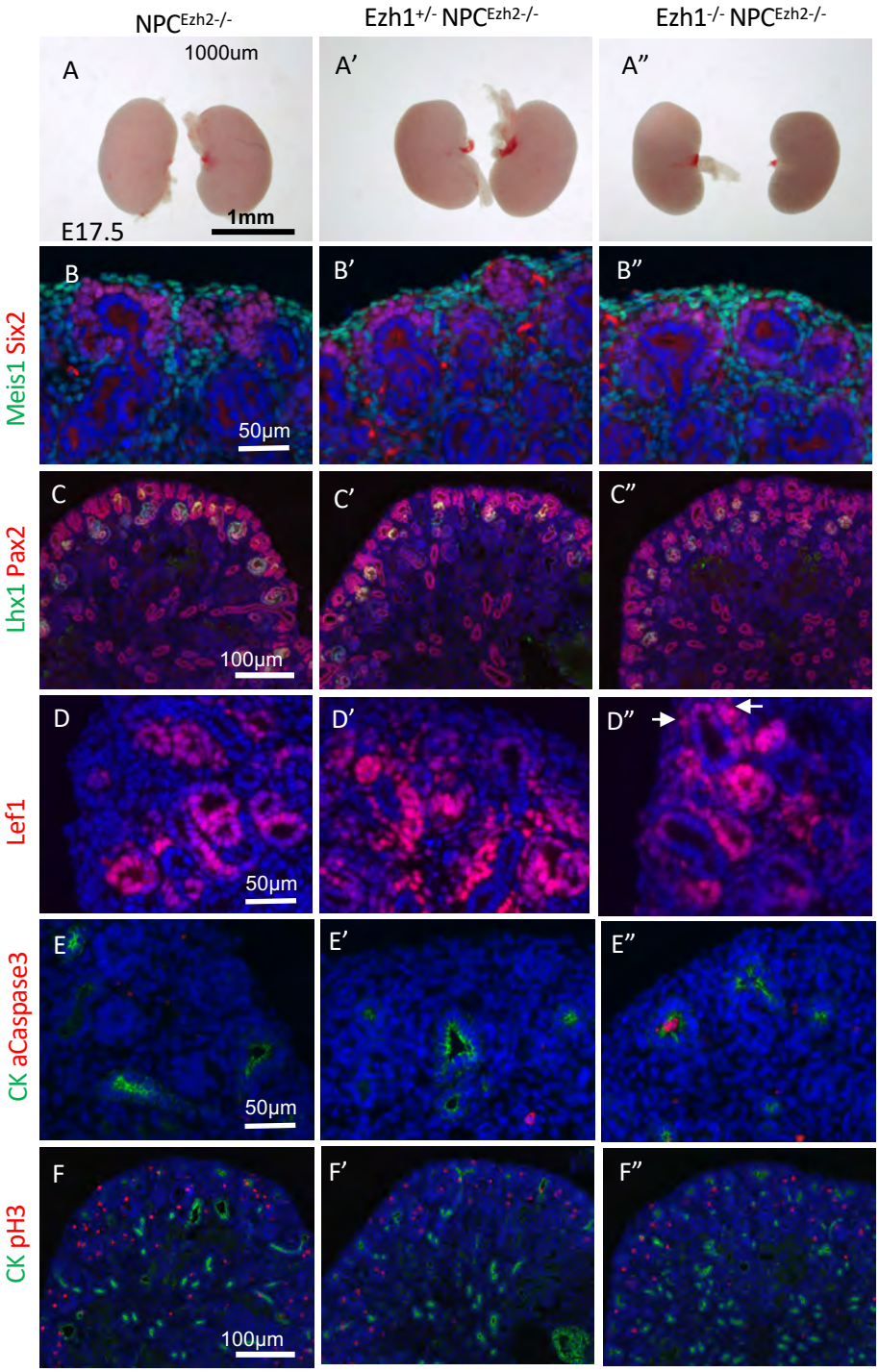
Supplemental Fig. 1 (S-1). Germline Ezh1$^{-/-}$ mice have normal nephrogenesis. (A-B') Gross view and histological H&E-stained sections. nz: nephrogenic zone, pt: proximal tubules, g: glomerulus. Section immunofluorescence using antibodies to Six2 and Lhx1 to label NPCs and nascent nephrons, respectively (C, C'); anti-neural cell adhesion molecule (NCAM) to label induced nephrons (D,D'); Note: NCAM also labels the medullary stroma; and staining for Lotus tetragonolobus lectin (LTL) to label the brush border of the proximal tubules (E,E').



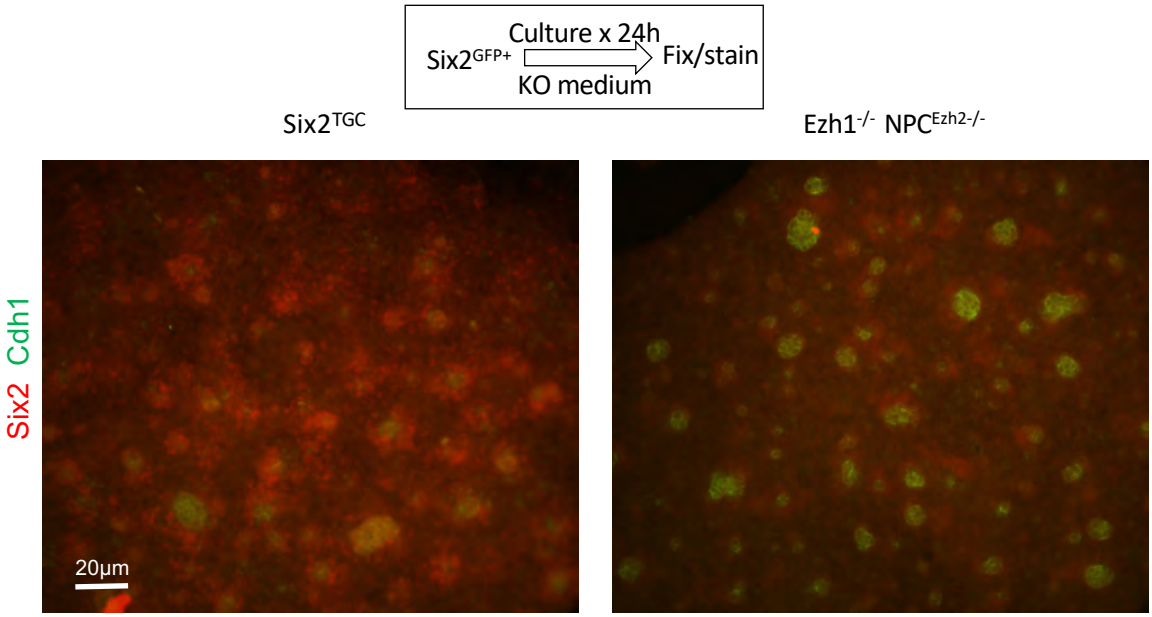
Supplemental Fig. 2 (Fig. S-2). Inactivation of Ezh1 and Ezh2 in NPCs has no effect on *Wnt9b* expression in the adjacent ureteric bud branches. Section ISH reveals UB-restricted expression of *Wnt9b* in all groups.



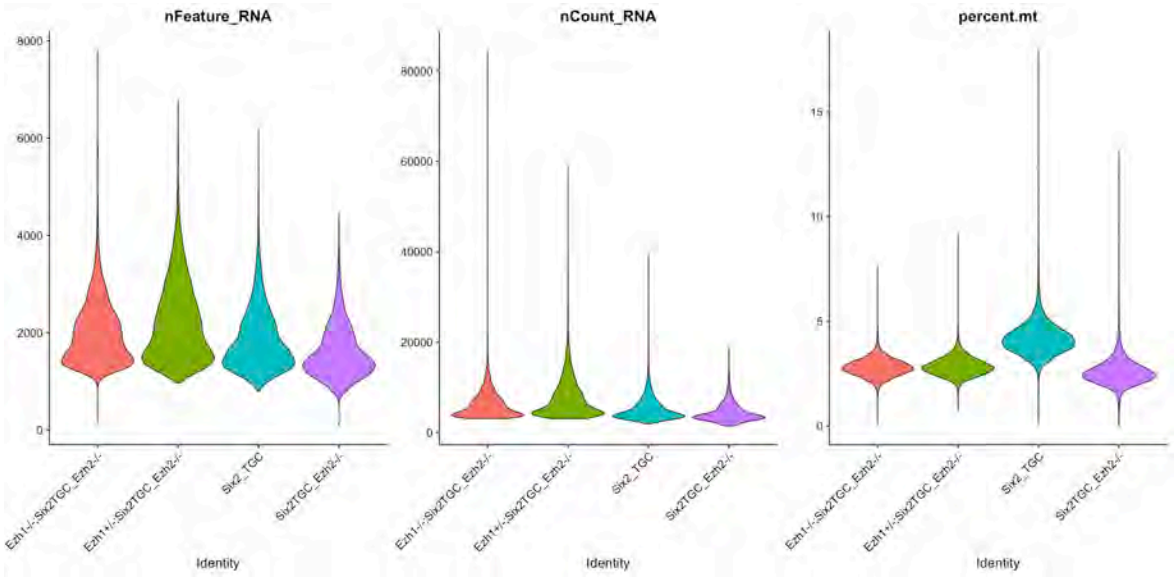
Supplemental Fig. 3 (Fig. S-3). Effect of Ezh1 and Ezh2 inactivation on nephrogenesis at E15.5. (A-A") Gross morphology. (B-B") Section H&E. (C-C") Six2^{+} cap mesenchyme is thinner and there are fewer Lhx1^{+} nascent nephrons in Ezh1/2 double mutant kidneys (inset). (D-D") There are fewer Jag1-expressing nascent nephrons (inset). (E-E") Active Caspase-3 staining (marker of apoptosis) is not altered across genotypes. (F-F") Phospho-S10-H3 staining, a marker of dividing cells, is decreased in double mutant kidneys.



Supplemental Fig. 4 (Fig. S-4). Effect of Ezh1 and Ezh2 inactivation on nephrogenesis at E17.5. (A-A") Gross morphology. (B-B") Six2^{+} cap mesenchyme is thinner in Ezh1/2 double mutant kidneys. (C-C") Fewer Lhx1^{+} nascent nephrons in double-mutant kidneys. (D-D") Lef1, a canonical Wnt-target, is expressed prematurely in NPCs of double mutant kidneys (arrows). (E-E") Active Caspase-3 staining (marker of apoptosis) is unaltered in Ezh1/2 mutant kidneys. (F-F") Phospho-S10-H3 staining, a marker of dividing cells, is decreased in double mutant kidneys.



Supplemental Fig. 5 (Fig. S-5). Inactivation of Ezh1 and Ezh2 promotes differentiation of cultured Six2GFP^{+} NPCs. E16.5 Six2GFP^{+} NPCs isolated from Six2^{TGC} (control) and Ezh1$^{-/-}$Six2$^{Ezh2-/-}$ kidneys were cultured for 24 hr in differentiation medium (see text) then stained with antibodies to Six2 and cell adhesion molecule E-cadherin.



Cell Counts before Sub-setting

```
table(Merged.ezh$orig.ident)
```

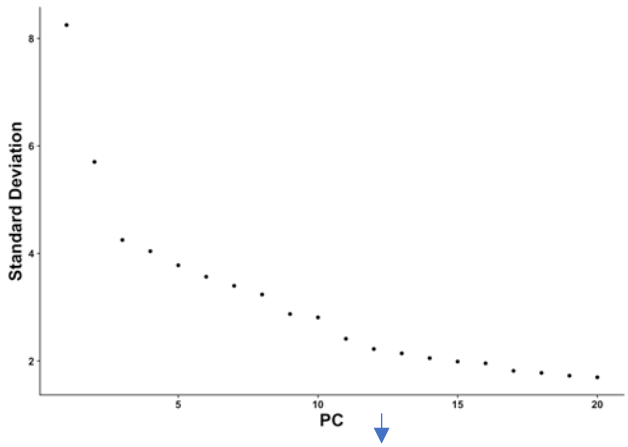
```
          Six2_TGC Six2TGC_Ezh2-/- Ezh1+/-;Six2TGC_Ezh2-/- Ezh1-/-;Six2TGC_Ezh2-/-
             10524            11382                    7512                    6574
```

Cell Counts after Sub-setting

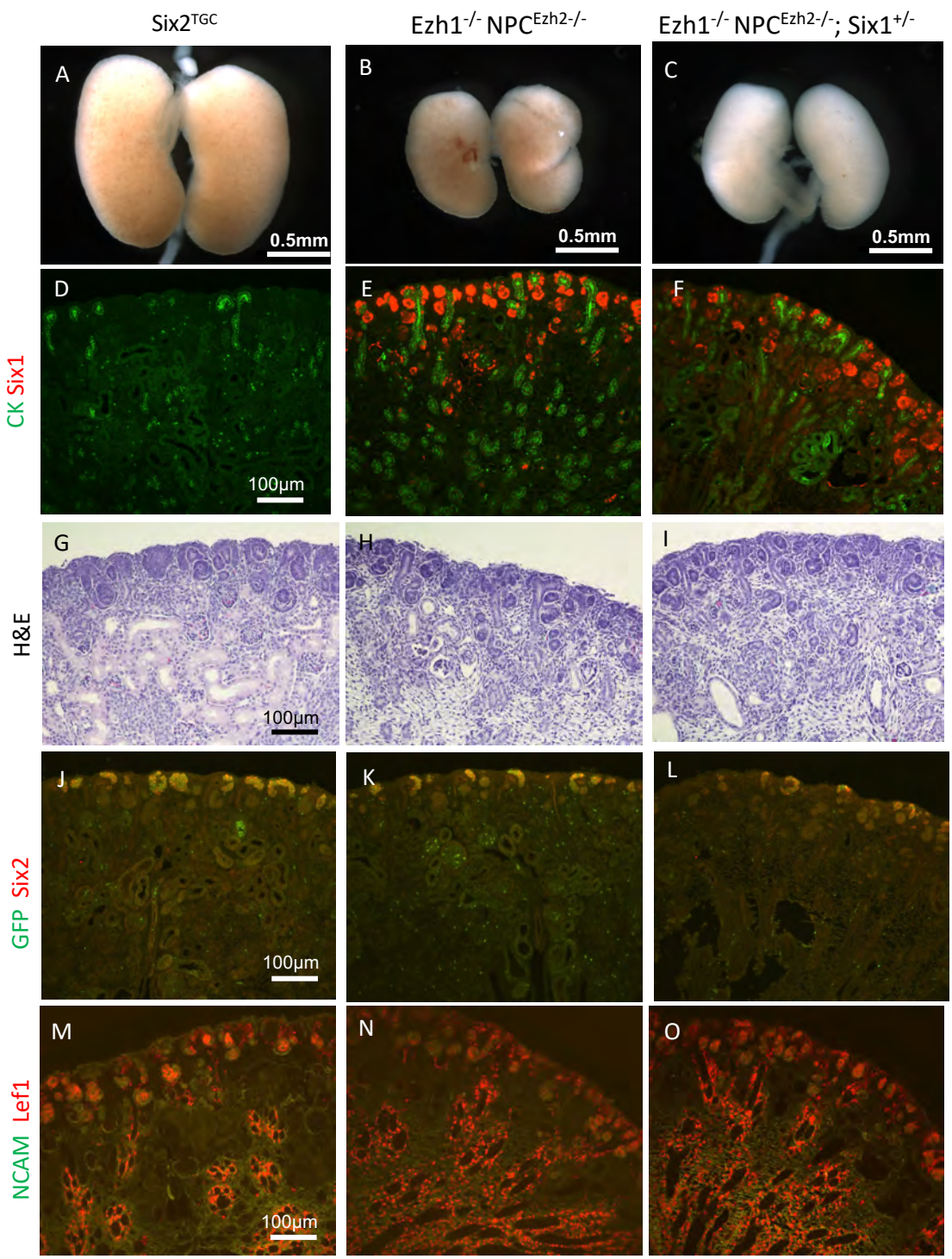
```
```{r}
Merged.ezh <- subset(Merged.ezh, subset = nFeature_RNA > 200 & nFeature_RNA < 5000 & percent.mt < 5) # default for nFeature_RNA <2500
```
```

```
> table(Merged.ezh$orig.ident)
```

```
          Six2_TGC Six2TGC_Ezh2-/- Ezh1+/-;Six2TGC_Ezh2-/- Ezh1-/-;Six2TGC_Ezh2-/-
              9258            11295                    7455                    6525
```



Supplemental Fig. 6 (Fig. S-6). Quality controls for scRNAseq studies.



Suppl. Fig. 7 (Fig. S-7). Six1 gene-dosage reduction fails to restore nephrogenesis in Ezh1$^{-/-}$NPC$^{Ezh2-/-}$ NPCs. (A-C) Gross view. (D-F) Section IF staining of Six1 and UB marker, cytokeratin, showing ectopic Six1 induction in Ezh1$^{-/-}$NPC$^{Ezh2-/-}$ NPCs and reduction of Six1 abundance in Ezh1$^{-/-}$NPC$^{Ezh2-/-}$;Six1$^{+/-}$ NPCs. (J-O) Section H&E and IF staining illustrating that Six1 gene-dosage reduction fails to restore Six2NPCs or nephrogenesis.